PATENT COOPERATION TREATY

PCT

REC'D 25 SEP 2003
WIPO PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference CG/P/143/WOD	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB02/03166	International filing date *(day/month/year)* 09.07.2002	Priority date *(day/month/year)* 10.07.2001
International Patent Classification (IPC) or both national classification and IPC C12R1/125		
Applicant THE SECRETARY OF STATE FOR DEFENCE et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
06.02.2003	23.09.2003
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Bassias, I Telephone No. +49 89 2399-8106

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB02/03166

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-14 as originally filed

Claims, Numbers

1-10 received on 16.08.2003 with letter of 12.08.2003

Drawings, Sheets

1/2-2/2 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-10
	No:	Claims	-
Inventive step (IS)	Yes:	Claims	1-10
	No:	Claims	-
Industrial applicability (IA)	Yes:	Claims	1-10
	No:	Claims	-

2. Citations and explanations

   see separate sheet

Re Item V

1. The amended claims filed with the letter of 12.08.2003 appear to be allowable under Articles 19(2) and 34(2)(b) PCT.

2. The present application relates to a recombinant asporogenic *Bacillus subtilis* strain in which the gene encoding sigma factor *spoIIAC* has been inactivated and additionally **at least three protease genes,** selected from *aprE, bpf, epr, mpr,* and *nprB* have been downregulated or inactivated.

3. A *B. subtilis* strain having mutations in *spoIIAC* and in at least three protease genes, selected from *aprE, bpf, epr, mpr,* and *nprB*, is not described in the prior art and thus the subject-matter of claims 1-10 fulfills the requirements of Article 33(2) PCT. The prior art teaches the use of asporogenic strains for production of heterologous proteins. Deletions of protease genes for producing target proteins is also known from the prior art. However, the combination of the inactivation of a sporulation gene, in particular *spoIIAC* and the mutation of **at least three protease genes** is neither known in the prior art nor considered obvious, since it cannot be foreseen that such a combination would produce a strain that can be successfully used for the production of heterologous proteins.
Consequently, claims 1-10 fulfill also the requirements of Article 33(3) PCT.

4. An adaptation of the description to the amended claims will be requested in the regional phase.

Claims

1. A recombinant microorganism comprising an asporogenic strain of *Bacillus subtilis* in which at least three genes which encodes a protease enzyme, selected from serine alkaline protease E (***aprE***), bacillopeptidase F (***bpf***), extracellular serine protease (***epr***), extracellular metalloprotease (*mpr*), extracellular neutral protease (***nprB***) or extracellular neutral metalloprotease (*nprE*) have been downregulated or inactivated, and wherein a gene encoding sigma factor *spoIIAC* has been inactivated such that the strain is asporogenic.

2. A recombinant microorganism according to claim 1 wherein all of the said protease enzyme genes are inactivated.

3. A recombinant microrganism according to any one of the preceding claims wherein the said protease enzyme genes are deleted.

4. A recombinant microorganism according to any one of the preceding claims wherein the gene encoding sigma factor *spoIIAC* is partially or totally deleted, and/or been subject to insertion mutagenesis.

5. A recombinant microorganism according to any one of the preceding claims which comprises a mutated form of *B. subtilis* 168.

6. A recombinant microorganism according to any one of the preceding claims, which has been transformed such that it contains a heterologous gene arranged such that the gene is expressed.

7. A recombinant microorganism according to claim 6 wherein said heterologous gene encodes an antigens or proteins useful in the production of a protective immune response to a pathogen.

8. A recombinant microorganism according to claim 7 wherein said heterologous gene encodes PA of *B. anthracis* or an immunogenic fragments or domains thereof, or a variant of any of these.

9. A recombinant microorganism according to claim 8 wherein said heterologous gene encodes PA of *B. anthracis* or one or more of domains 1 and 4 or protective regions thereof, of the full length sequence.

10. A method for producing a target protein, said method comprising transforming a recombinant microorganism according to any one of claims 1 to 9 with a nucleotide sequence which encodes said protein, culturing said transformed strain and recovering said target protein from the culture.